

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed : June 7, 1995
Page 2

Please add the following new claims 101-125:

--101. (New) A composition which comprises:

- a) a conjugate comprising (i) a GD3 lactone ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base and (ii) Keyhole Limpet Hemocyanin or a derivative thereof;
- b) a saponin derivable from the bark of a Quillaja saponaria Molina tree; and
- c) a pharmaceutically acceptable carrier;

K1
the relative amounts of such conjugate and such saponin being effective to stimulate or enhance production in a subject of an antibody to GD3,

wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin or the derivative thereof through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin or the derivative thereof, wherein the C-4 carbon is present in a CH_2 group; and

wherein the derivative comprises Keyhole Limpet Hemocyanin linked to an immunological adjuvant, a non-ionic block copolymer, or a cytokine.--

--102. (New) The composition of claim 101, wherein the saponin

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed : June 7, 1995
Page 3

is QS-21.--

--103. (New) The composition of claim 101, wherein the amount of the conjugate is an amount between about 1 μ g and about 200 μ g.--

--104. (New) The composition of claim 103, wherein the amount of the conjugate is between 10 μ g and 90 μ g.--

--105. (New) The composition of claim 103, wherein the amount of the conjugate is between 10 μ g and 70 μ g.--

--106. (New) The composition of claim 103, wherein the amount of the conjugate is between 10 μ g and 50 μ g.--

--107. (New) The composition of claim 101, wherein the amount of the saponin is an amount between about 10 μ g and about 200 μ g.--

KL
W
--108. (New) The composition of claim 107, wherein the amount of the saponin is about 100 μ g.--

--109. (New) The composition of claim 107, wherein the amount of the saponin is about 200 μ g.--

--110. (New) The composition of claim 101, wherein the GD3 lactone:Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1.--

--111. (New) A composition which comprises:

a) a conjugate comprising (i) a GD3 lactone ganglioside derivative which comprises an unaltered

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed : June 7, 1995
Page 4

oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base and (ii) Keyhole Limpet Hemocyanin or a derivative thereof;

b) a saponin derivable from the bark of a Quillaja saponaria Mollina tree, wherein the saponin is QS-21; and

c) a pharmaceutically acceptable carrier;

wherein the conjugate is present in an amount of between about 10 μ g and about 50 μ g, the amount of the saponin is about 100 μ g and the GD3 lactone:Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, and wherein the amount of such conjugate and such saponin is effective to stimulate or enhance production in a subject of an antibody to GD3;

and wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin or the derivative thereof through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin or the derivative thereof, wherein the C-4 carbon is present in a CH_2 group; and

wherein the derivative comprises Keyhole Limpet Hemocyanin linked to an immunological adjuvant, a non-ionic block copolymer or a cytokine.--

--112. (New) A method of treating a subject afflicted with melanoma which comprises administering to said subject an amount of the composition of claim 111 effective to stimulate or enhance production of an antibody to GD3 in

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed : June 7, 1995
Page 5

the subject and to thereby treat said melanoma in said subject.--

--113. (New) A method of stimulating or enhancing production of an antibody to GD3 in a subject which comprises administering to the subject an effective amount of a composition which comprises:

- a) a conjugate comprising (i) a GD3 lactone ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base and (ii) Keyhole Limpet Hemocyanin or a derivative thereof;
- b) a saponin derivable from the bark of a Quillaja saponaria Molina tree; and
- c) a pharmaceutically acceptable carrier;

K1
the relative amounts of such conjugate and such saponin being effective to stimulate or enhance production in a subject of an antibody to GD3,

wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin or the derivative thereof through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin or the derivative thereof, wherein the C-4 carbon is present in a CH_2 group, so as to thereby stimulate or enhance production in the subject of the antibody to GD3.--

--114. (New) A method of treating a cancer in a subject which

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed : June 7, 1995
Page 6

comprises administering to the subject an effective cancer treating amount of a composition which comprises:

a) a conjugate comprising (i) a GD3 lactone ganglioside derivative which comprises an altered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base and (ii) Keyhole Limpet Hemocyanin, or a derivative thereof;

b) a saponin derivable from the bark of a Quillaja saponaria Molina tree; and

c) a pharmaceutically acceptable carrier;

the relative amounts of such conjugate and such saponin being effective to stimulate or enhance production in a subject of an antibody to GD3,

KL
W
wherein, in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin or the derivative thereof through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin or the derivative thereof, wherein the C-4 carbon is present in a CH_2 group, so as to stimulate or enhance production of an antibody to GD3 in the subject and thereby treat the cancer.--

--115. (New) The method of claim 114, wherein the cancer is of epithelial origin.--

--116. (New) The method of claim 114, wherein the cancer is of neuroectodermal origin.--

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed : June 7, 1995
Page 7

- 117. (New) The method of claim 116, wherein the cancer of neuroectodermal origin is a melanoma.--
- 118. (New) The method of claim 113 or 114, wherein the administering is effected at two or more sites.--
- 119. (New) The method of claim 118, wherein the administering is effected at three sites.--
- 120. (New) The method of claim 113 or 114, wherein the composition is administered subcutaneously to said subject.--
- 121. (New) The method of claim 120, wherein the composition is administered to said subject at two-week intervals.--
- KI
4 --122. (New) The method of claim 120, wherein the composition is initially administered to said subject at weekly intervals.--
- 123. (New) The method of claim 113 or 114, wherein the composition to be administered is prepared prior to administration to the subject by mixing the conjugate and the saponin.--
- 124. (New) The method of claim 123, wherein the conjugate and the saponin are mixed on the day of administration to the subject.--
- 125. (New) A method of delaying recurrence of melanoma in subjects at risk of relapse of melanoma which comprises administering to the subject an effective melanoma treating amount of a composition which comprises:

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed : June 7, 1995
Page 8

- a) a conjugate comprising (i) a GD3 lactone ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base and (ii) Keyhole Limpet Hemocyanin or a derivative thereof;
- b) a saponin derivable from the bark of a Quillaja saponaria Molina tree; and
- c) a pharmaceutically acceptable carrier;

the relative amounts of such conjugate and such saponin being effective to stimulate or enhance production in a subject of an antibody to GD3,

10
m
wherein in the conjugate the ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin or the derivative thereof through a C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative to an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin or the derivative thereof, wherein the C-4 carbon is present in a CH_2 group, so as to stimulate or enhance production of an antibody to GD3 in said subject and thereby delay recurrence of melanoma in said subject at risk of relapse of said melanoma,

wherein the derivative comprises Keyhole Limpet Hemocyanin linked to an immunological adjuvant, a non-ionic block copolymer, or a cytokine. -
